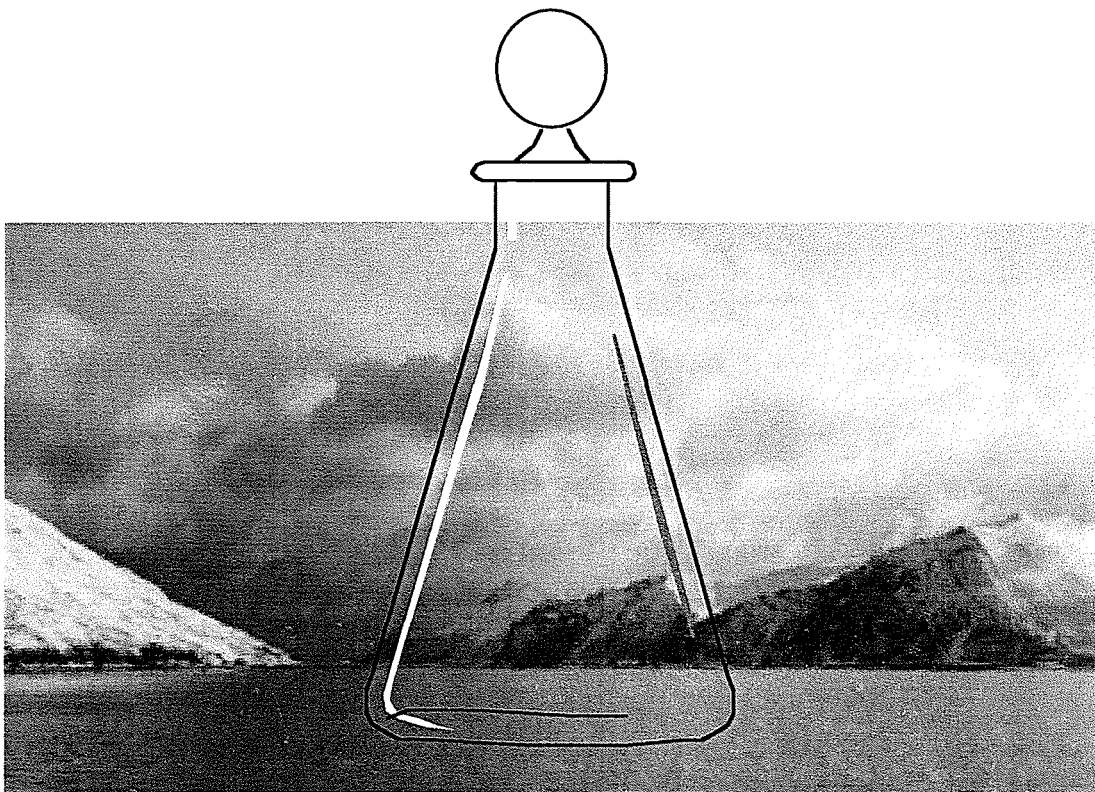


**National Status and Trends Program**  
for Marine Environmental Quality

**NOAA/NRC Second Intercomparison for  
Nutrients in Seawater**



Dutch Harbor, Aleutian Islands, Alaska, 1953. Rear Admiral H. D. Nygren, NOAA Corps (ret.) (NOAA Photo Collection, NOAA Central Library)

Silver Spring, Maryland  
November 2002



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Center for Coastal Monitoring and Assessment  
National Centers for Coastal Ocean Science  
National Ocean Service

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#### Disclaimer

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## **NOAA/NRC Second Intercomparison for Nutrients in Seawater**

**Scott Willie and Vincent Clancy**

**Institute for National Measurement Standards  
National Research Council of Canada  
Ottawa, Ontario, Canada**



November 2002

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Department of Commerce

Donald L. Evans  
Secretary

National Oceanic and  
Atmospheric Administration

Scott B. Gudes  
Acting Under Secretary

National Ocean Service

Margaret Davidson  
Acting Assistant Administrator

---

## **Abstract**

An intercomparison exercise was undertaken to assess the current capabilities of a group of laboratories to quantitate orthophosphate, silicate, nitrite and nitrite + nitrate in a seawater sample. This is the second such exercise sponsored by the NOAA Center for Coastal Monitoring and Assessment (CCMA) and coordinated by the Institute for National Measurement Standards of the National Research Council of Canada. Two seawater samples; one from Pensacola Sound FL, and a proposed seawater certified reference material ( MOOS-1), were distributed to thirty-one laboratories. Twenty-four laboratories submitted data. Methodologies were not prescribed to the participants, however, all reported results were obtained based on traditional colorimetric procedures. Generally, satisfactory agreement among participants was achieved with results within ten percent of the assigned mean values.

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This is the second intercomparison exercise for the determination of nutrients in seawater organized by the National Research Council of Canada (NRC) on behalf of the Center for Coastal Monitoring and Assessment of the National Oceanic and Atmospheric Administration (NOAA), National Centers for Coastal Ocean Sciences (NCCOS). The purpose of this exercise was to assess the capabilities of a number of NOAA and other laboratories to analyse seawater for orthophosphate, dissolved silica, nitrite and total oxidised nitrogen (nitrite + nitrate). Laboratories that submitted results in the first exercise, as well as several EPA, laboratories were invited to participate. NRC was asked to coordinate the sample distribution, collect the results, analyze the data as well as prepare and distribute a final report.

The test materials distributed by NRC were:

#### **Nutrient 2002**

A seawater sample was collected at the laboratory of the USEPA Gulf Ecology Division in Gulf Breeze, Florida on May 13, 2001. Water was taken from the Pensacola Sound at a depth of 3 m using a diaphragm pump and passed through a progression of filters before final filtration through a 0.05 µm cartridge into 10L polyethylene jugs. The water was shipped to NRC in Ottawa and immediately sent for sterilization via gamma irradiation at MDS Nordion in Laval, Quebec. Nutrient 2002 is a blend of Pensacola Sound seawater and MOOS-1.

#### **MOOS-1**

A proposed certified reference material for nutrients in seawater. This water was collected at Lat. 47.062833 °N, Long. 59.982333 °W, off the northern tip of Cape Breton Island. The water was collected from a depth of about 200 meters using a rosette containing 22 Niskin bottles of about 10L each. Two casts were made. The contents of each Niskin were transferred, by means of a peristaltic pump, through a 0.05 µm cartridge filter into 50L carboys. The water was returned to the NRC laboratories in Ottawa, Ontario and homogenized in a 400L tank. Fifty mL subsamples were aliquoted into precleaned plastic bottles, sealed, and gamma irradiated with 25 kGy. The water was collected June 24, 1996, bottled July 11 & 12, and irradiated July 16, 1996. It was considered insufficiently homogeneous and, in April 2001, the samples were reblended and bottled.

Homogeneity testing of the samples was performed at NRC. The participating laboratories were each sent a 50 ml bottle of the unknown sample Nutrient 2002 and a bottle of MOOS-1 and requested to perform duplicate analyses on each of the bottles. Laboratories were supplied with indicative values for MOOS-1 derived from the previous intercomparison. The participants were also sent a data file in which to record their results and analytical procedures.

All concentrations are expressed in micromoles per liter.

The prepared samples were mailed to the participating laboratories listed in Appendix A in June, 2002, with the deadline for receipt of results set for September 8, 2002. Twenty-four sets of results were received. Sequential numbers were assigned to each responding laboratory upon receipt of its data. Laboratory number 25 was assigned to NRC. The submitted data are listed in Appendix B.

The method of robust statistics was used to calculate the assigned mean and standard deviation from the data submitted by the participating laboratories. Robust statistics is a recommended method of summarizing results when a small proportion of outliers is suspected. The assigned concentrations are listed above the appropriate graph of results for each sample. Replicate data are plotted in the graphs with a solid horizontal line representing the assigned mean and a shaded area representing the corresponding range. A histogram of the sample data is also shown in the second graph for each analyte.

The z-scoring system is an accepted method used in intercomparison exercises to assess bias. This is accomplished by comparison of the bias estimate for each analyte with a target value for standard deviation. The bias estimate is calculated from the difference between the laboratory mean ( $x_i$ ) and the accepted (or assigned) mean ( $\bar{x}$ ). The z-score is calculated by dividing the bias estimate by the target value for standard deviation ( $\sigma_{\text{target}}$ ). A table of z scores is listed for each analyte.

$$z = \frac{(x_i - \bar{x})}{\sigma_{\text{target}}}$$

$ Z  \leq 2$	satisfactory
$ Z  \geq 2$	questionable

A target standard deviation of 5% was arbitrarily chosen for scoring laboratory performance and calculating z scores. It should be emphasized that it is the responsibility of the participating laboratory to ascertain their own accuracy requirements.

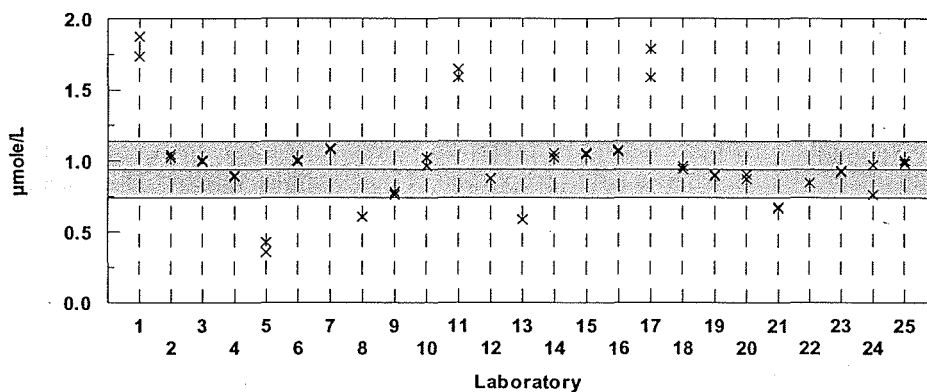
Also included for each analyte are Youden or two sample plots. These graphs of a laboratory's mean result for MOOS-1 plotted versus the reported mean for the unknown sample can give useful information when the analyte concentrations of the two samples are similar. If non-systematic or random errors occur, the results would be expected to group at random over the area of the graph. If, however, systematic errors occur (e.g., a high or low result for both the CRM and the unknown) a predominance of points would be expected to group about a line running from the origin through the intersection of the two means.

Appendix C summarizes the analytical procedures reported by the participating laboratories.

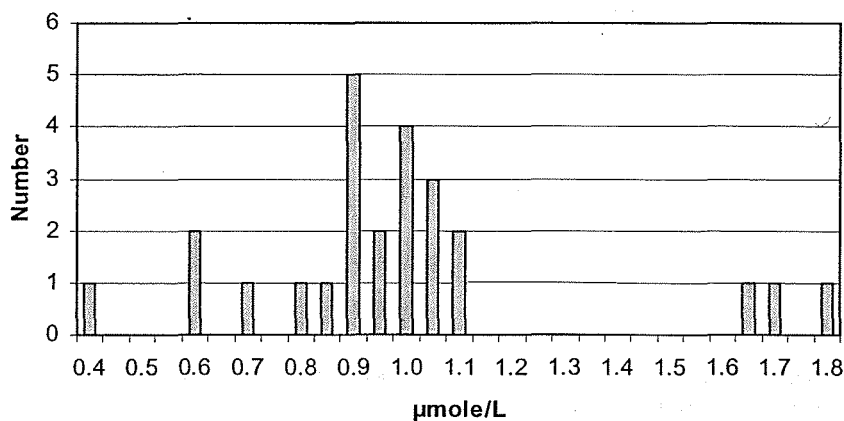
**Phosphate**

**Nutrient 2002**

Assigned Value :  $0.94 \pm 0.20$   $\mu\text{mole/L}$



**Histogram for Nutrient 2002**



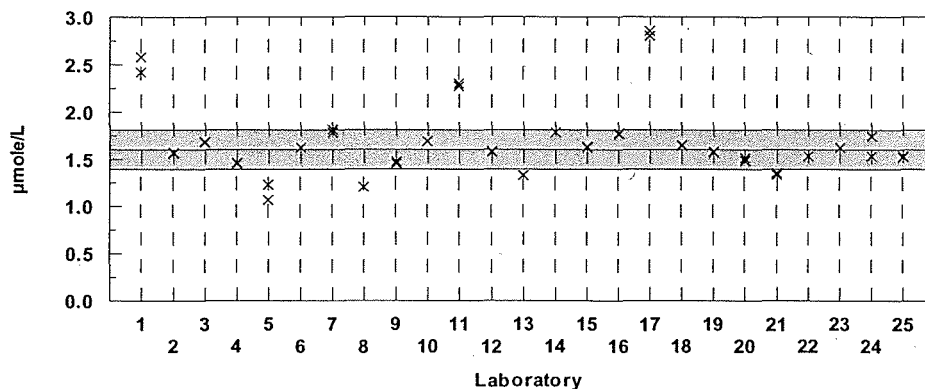
**Z scores for Nutrient 2002**

Lab	z score	Lab	z score
1	8.6	13	-3.6
2	0.9	14	1.0
3	0.6	15	1.1
4	-0.5	16	1.3
5	-5.6	17	7.4
6	0.6	18	0.1
7	1.5	19	-0.4
8	-3.4	20	-0.5
9	-1.7	21	-2.7
10	0.5	22	-1.0
11	6.7	23	-0.1
12	-0.7	24	-0.8
		25	0.5

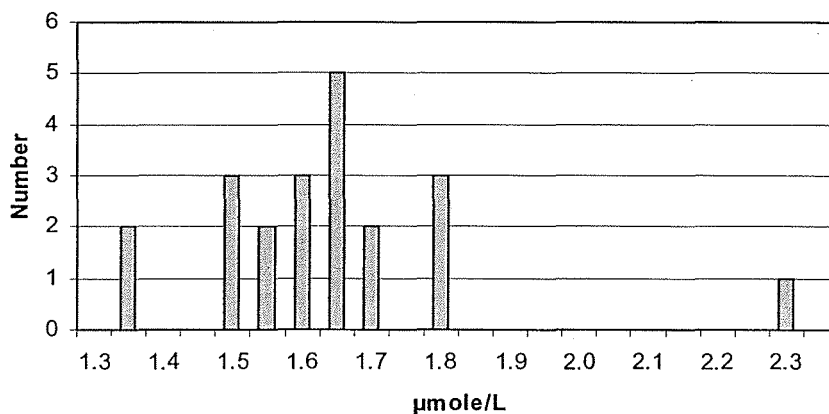


# MOOS-1

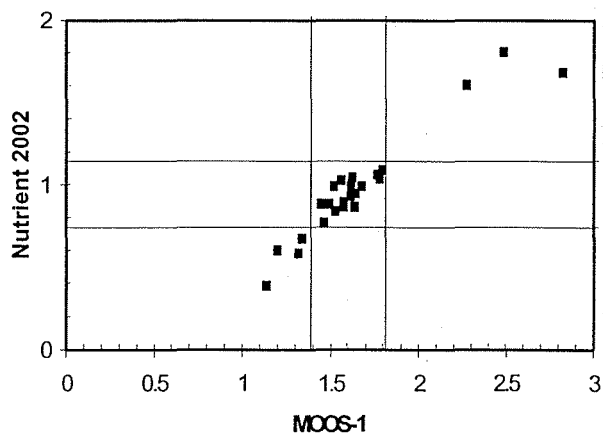
Assigned Value :  $1.60 \pm 0.21$   $\mu\text{moles/L}$



## Histogram for MOOS-1



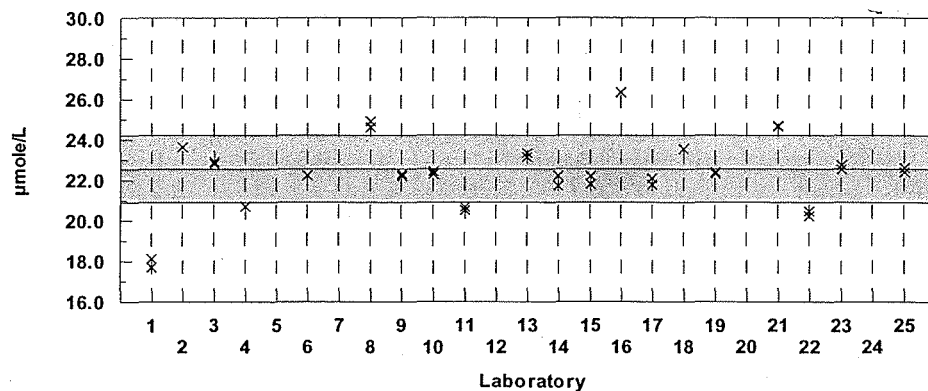
## Youden Plot for Orthophosphate



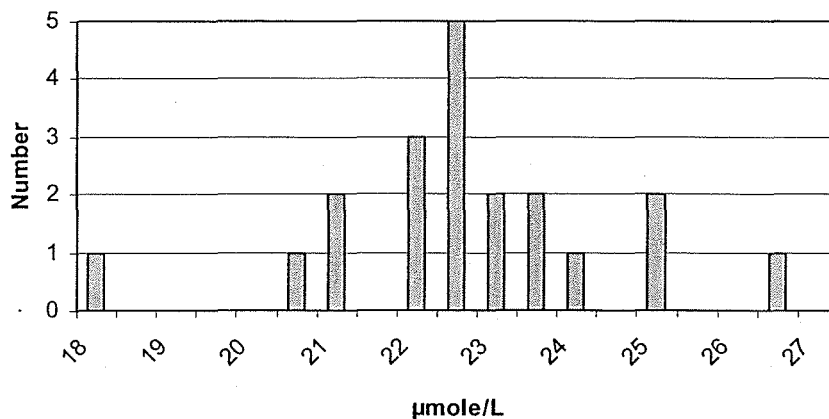
Silicate

Nutrient 2002

Assigned Value :  $22.5 \pm 1.6$   $\mu\text{moles/L}$



Histogram for Nutrient 2002



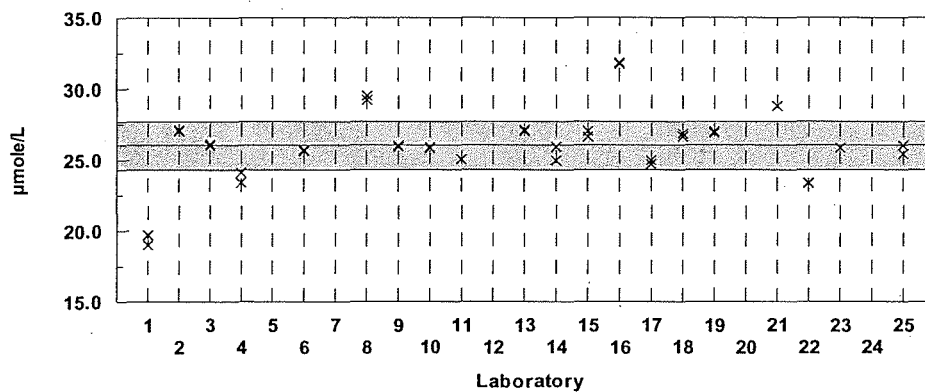
Z scores for Nutrient 2002

Lab	z score
1	-4.1
2	1.0
3	0.3
4	-1.6
5	-0.3
8	2.0
9	-0.2
10	-0.1
11	-1.7
13	0.6

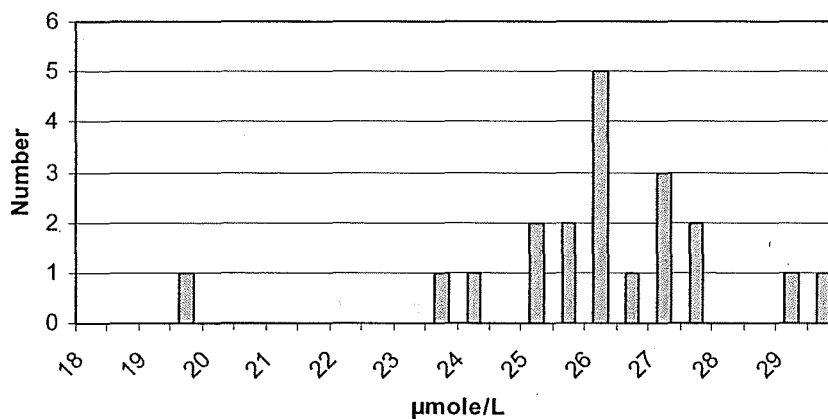
Lab	z score
14	-0.5
15	-0.4
16	3.4
17	-0.5
18	0.9
19	-0.1
21	1.9
22	-1.9
23	0.1
25	0.0

**MOOS-1**

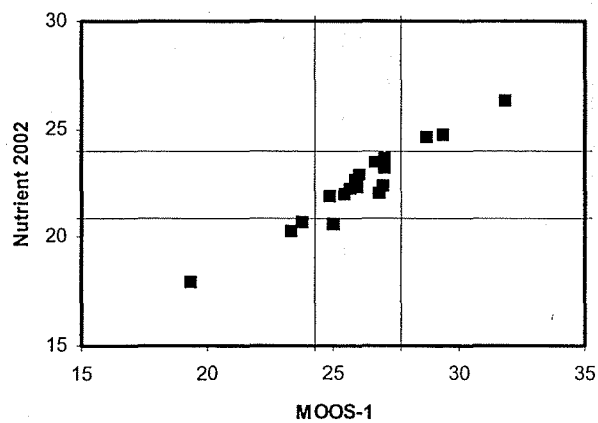
Assigned Value :  $26.0 \pm 1.7$   $\mu\text{moles/L}$



**Histogram for MOOS-1**



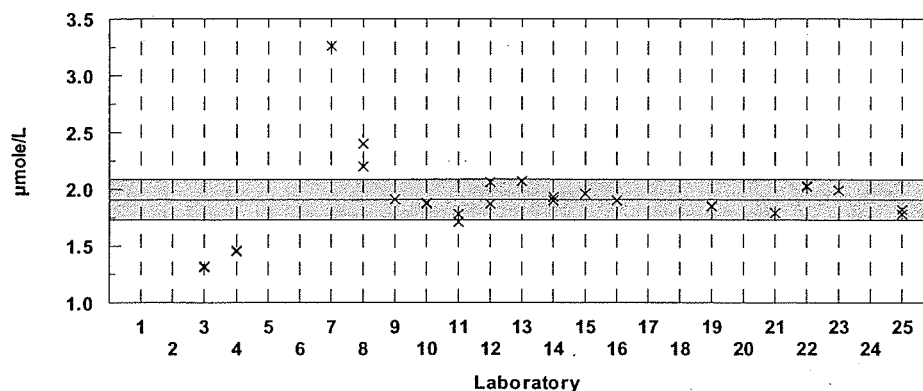
**Youden Plot for Silicate**



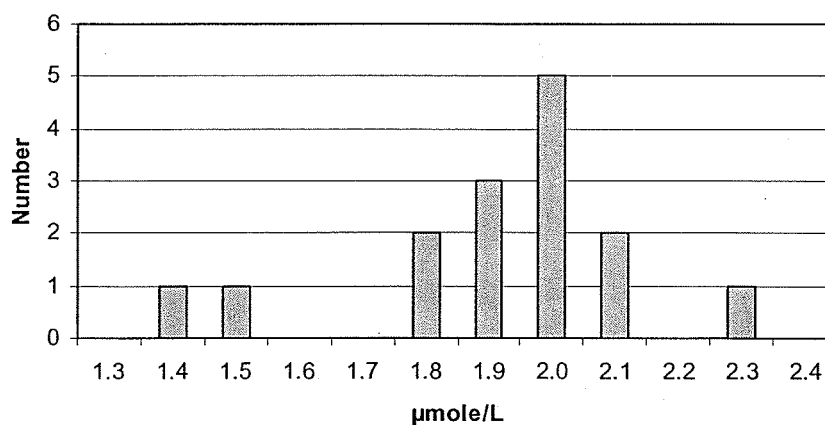
Nitrite

# Nutrient 2002

Assigned Value :  $1.91 \pm 0.18$   $\mu\text{moles/L}$



## Histogram for Nutrient 2002

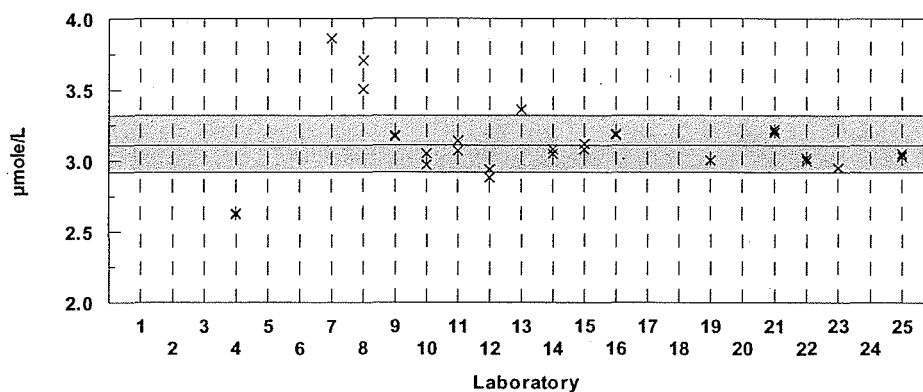


## Z scores for Nutrient 2002

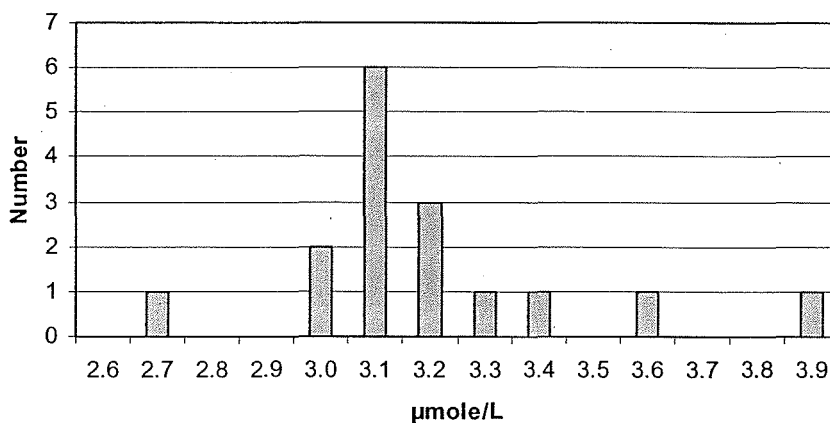
Lab	z score	Lab	z score
3	-6.2	13	1.7
4	-4.8	14	0.1
7	14.1	15	0.5
8	4.1	16	-0.1
9	0.0	19	-0.6
10	-0.4	21	-1.2
11	-1.7	22	1.2
12	0.6	23	0.8

# MOOS-1

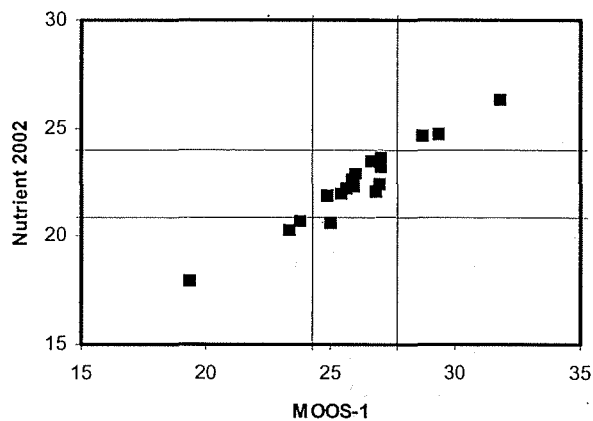
Assigned Value :  $3.10 \pm 0.19$   $\mu\text{moles/L}$



## Histogram for MOOS-1

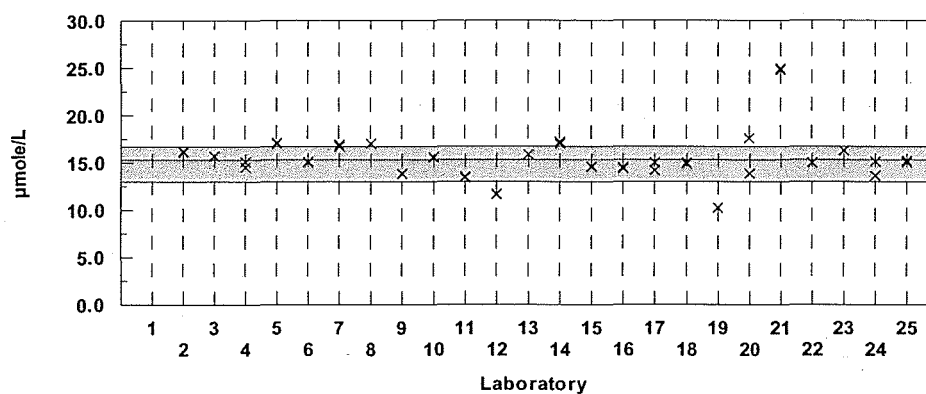


## Youden Plot for Nitrite

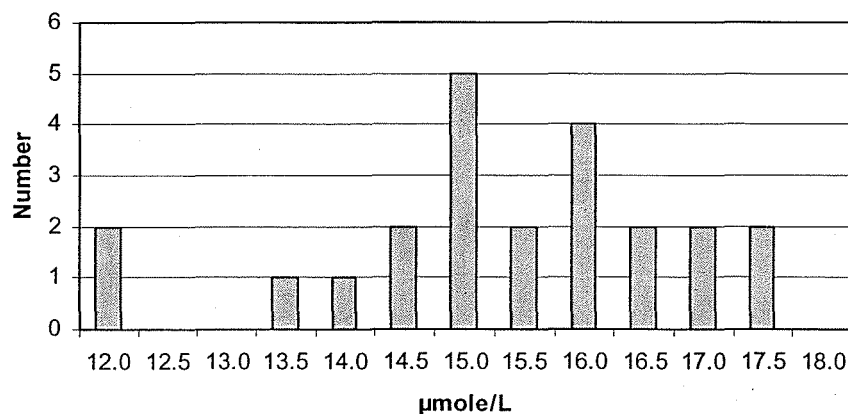


# Nutrient 2002

Assigned Value :  $15.3 \pm 1.4$   $\mu\text{moles/L}$



## Histogram for Nutrient 2002

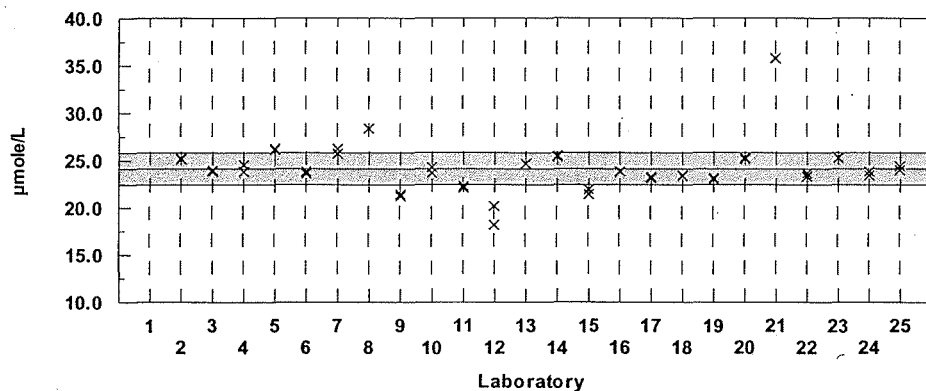


## Z scores for Nutrient 2002

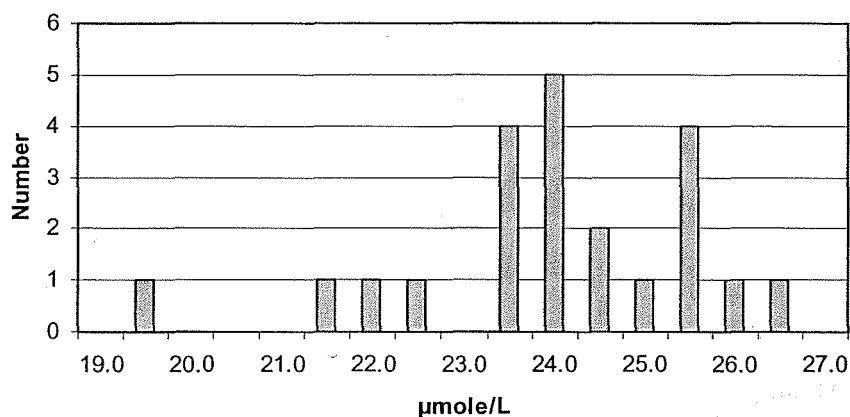
Lab	z score	Lab	z score
2	1.1	14	2.4
3	0.6	15	-1.0
4	-0.6	16	-1.2
5	2.3	17	-0.9
6	-0.3	18	-0.5
7	2.0	19	-6.8
8	2.3	20	0.5
9	-2.0	21	12.5
10	0.4	22	-0.4
11	-2.4	23	1.3
12	-4.8	24	-1.2
13	0.8	25	-0.2

# MOOS-1

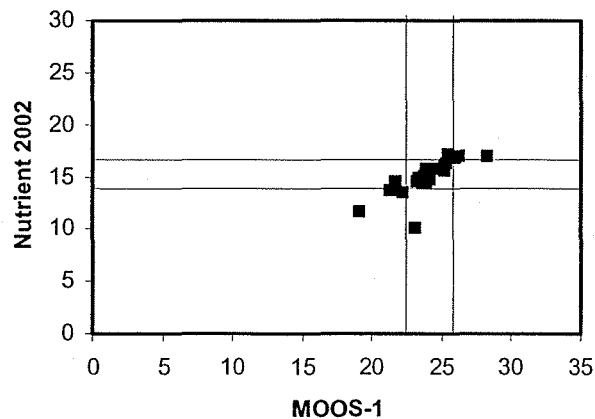
Assigned Value :  $24.1 \pm 1.7$   $\mu\text{moles/L}$



## Histogram for MOOS-1



## Youden Plot for Nitrite + Nitrate



# Discussion

In 1999, NRC coordinated a similar intercomparison for nutrients in seawater. Two fifty ml samples of MOOS-1 were sent to thirty laboratories, many of the same participants in this study. The submitted data indicated that interbottle inhomogeneity was larger than desired if MOOS-1 was to be a useful CRM. A specific experiment aimed to hasten and expose potential instability in Nutrient 2002 was undertaken to ensure this problem did not reoccur. An isochronous experiment was conducted by subjecting several bottles of Nutrient 2002 to a temperature of 40°C for periods up to 40 days. The results from this experiment are shown in Table 1 compared to the initial orthophosphate analysis of Nutrient 2002 performed in November 2001.

**Table 1**  
**Isochronous Study of Nutrient 2002**

	20-Nov-01	11-Jan-02		
		no heat	33 days	40 days
Nitrite, $\mu\text{M}$	$1.80 \pm 0.01$	$1.87 \pm 0.01$	$1.87 \pm 0.01$	$1.88 \pm 0.01$
Phosphate, $\mu\text{M}$	$1.01 \pm 0.04$	$0.98 \pm 0.03$	$1.05 \pm 0.08$	$1.04 \pm 0.04$

Additionally, NRC analyzed many of the Nutrient 2002 samples for orthophosphate prior to distribution to the participants. The relative standard deviation (RSD) of these measurements was 3.6%. The RSD on the raw data for the submitted phosphate results in Nutrient 2002 was 33% (21% following rejection of outliers). This represents the highest imprecision of all analytes in this study. For comparative purposes, the bottle to bottle inhomogeneity of orthophosphate in the proposed CRM MOOS-1 was <1%.

The majority of results for silicate, nitrite and nitrite + nitrate were within 5 to 10% of their assigned means with calculated RSD's lower for MOOS-1 than the unknown sample.

With very few exceptions, it is noteworthy that a laboratory that submitted a result outside the assigned range for Nutrient 2002 also submitted a similarly biased result for the same analyte in MOOS-1, as illustrated in the Youden Plots. For all analytes, the preponderance of data fall along a straight line 45° from the origin. Nonsystematic biases due to random errors or unstable samples would result in data points deviating from this pattern.

Laboratories were asked to report on the data form if saline standards were used for calibration. The results are summarized in Table 2. The majority of participants used standards prepared in seawater or NaCl. One laboratory used a correction factor and another performed calibration using the method of standard additions.

**Table 2**  
**Use of Saline Standards**

Lab	Saline Standards	Lab	Saline Standards
1	yes	14	NaCl
2	yes	15	no
3	yes	16	yes
4	no	17	yes
5	no	18	yes
6	NaCl	19	yes
7	no	20	no
8	no	21	yes
9	no	22	correction
10	yes	23	no
11	std add	24	yes
12	no	25	NaCl
13	no		



Since a criterion for evaluating intralaboratory precision was not assigned for this study, an evaluation of laboratory precision was not attempted. Most laboratories performed the analysis of duplicates on one day, however, laboratories 4, 11, 14 and 24 performed duplicate analysis of the analytes on separate days

## CONCLUSIONS

The results from this exercise suggest the earlier homogeneity problem which was identified in the first intercomparison exercise has been overcome, although the orthophosphate data indicates a larger interlaboratory spread of results than expected.

Results for the determination of silicate, nitrite and nitrite + nitrate in the distributed seawater samples were acceptable for the majority of the participants and generally deviated  $< \pm 10\%$  from the assigned mean. All laboratories used methodology based on colorimetric principles.

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- Statistical methods for use in proficiency testing by interlaboratory comparisons. Draft version ISO/DIS 13528.
- NOAA/NRC Intercomparison for Nutrients in Seawater, S. Willie and V. Clancy, NOAA Technical Memorandum NOS NCCOS CCMA 143, 2000.

## ACKNOWLEDGEMENTS

The authors thank John Macauley of the USEPA Gulf Ecology Division in Gulf Breeze Florida for the use of his laboratory facilities to collect seawater.

**Appendix A**
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Management  
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Jacksonville, FL, USA

Fisheries and Oceans Canada  
Bedford Institute of Oceanography  
Marine Nutrient Lab  
Dartmouth, NS, Canada

Fisheries and Oceans Canada  
Institut Maurice-Lamontagne  
Mont-Joli, QC, Canada

Fisheries and Oceans Canada  
Institute of Ocean Science  
Sidney, BC Canada

Fisheries and Oceans Canada,  
Northwest Atlantic Fisheries Centre  
St. John's, NF, Canada

Florida Dept. of Environmental Protection  
Chemistry Section  
Tallahassee, FL, USA

Florida State University  
Dept of Oceanography  
Tallahassee, FL, USA

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Seattle, WA, USA

Lumcon  
Chauvin, LA, USA

Miami-Dade DERM Laboratory  
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NYCDEP  
Chemistry Lab  
Wards Island, NY, USA

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Atlantic Oceanographic & Meteorological Laboratory  
Rickenbacker Causeway  
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USEPA  
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University of Connecticut  
Environmental Research Institute  
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Center for Ecology and Environmental Technology  
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Center for Environmental Science  
Horn Point Laboratory  
Cambridge, MD, USA

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RSMAS/ Dept. of MBF  
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Plymouth Environmental Research Centre  
Plymouth, UK

University of Washington  
School of Oceanography  
Seattle, WA, USA

## Laboratory Results for Phosphate

Nutrient 2002			MOOS-1		
$\mu\text{mole/L}$			$\mu\text{mole/L}$		
1	1.73	1.87	1	2.41	2.57
2	1.02	1.04	2	1.56	1.57
3	1.00	0.99	3	1.67	1.68
4	0.884	0.892	4	1.46	1.45
5	0.42	0.35	5	1.22	1.06
6	0.99	1.00	6	1.62	1.62
7	1.09	1.08	7	1.78	1.81
8	0.6	0.6	8	1.2	1.2
9	0.76	0.78	9	1.45	1.47
10	1.022	0.964	10	1.682	1.678
11	1.58	1.64	11	2.26	2.29
12	0.87	0.87	12	1.58	1.57
13	0.58	0.58	13	1.32	1.32
14	1.05	1.02	14	1.78	1.78
15	1.05	1.04	15	1.62	1.63
16	1.07	1.06	16	1.76	1.77
17	1.78	1.58	17	2.85	2.80
18	0.94	0.96	18	1.64	1.65
19	0.901	0.892	19	1.568	1.581
20	0.9032	0.8709	20	1.48	1.51
21	0.66	0.67	21	1.35	1.33
22	0.84	0.84	22	1.53	1.53
23	0.92	0.93	23	1.62	1.62
24	0.97	0.76	24	1.74	1.53
25	0.98	1.00	25	1.53	1.52

## Laboratory Results for Silicate

Nutrient 2002			MOOS-1		
$\mu\text{mole/L}$			$\mu\text{mole/L}$		
1	18.12	17.69	1	18.99	19.69
2	23.6	23.6	2	27	27.1
3	22.9	22.8	3	26.0	26.1
4	20.7	20.7	4	23.4	24.1
5			5		
6	22.2	22.2	6	25.7	25.6
7			7		
8	24.9	24.6	8	29.5	29.2
9	22.3	22.2	9	25.9	26
10	22.28	22.42	10	25.88	25.81
11	20.66	20.5	11	25	25
12			12		
13	23.1	23.3	13	27.1	27
14	22.2	21.7	14	24.9	25.9
15	22.2	21.8	15	27	26.6
16	26.29	26.28	16	31.83	31.73
17	22.05	21.74	17	24.69	24.95
18	23.5	23.5	18	26.6	26.8
19	22.39	22.31	19	26.99	26.87
20			20		
21	24.68	24.63	21	28.73	28.73
22	20.43	20.19	22	23.37	23.33
23	22.73	22.53	23	25.84	25.83
24			24		
25	22.4	22.6	25	25.4	26

## Laboratory Results for Nitrite

Nutrient 2002			MOOS-1		
$\mu\text{mole/L}$			$\mu\text{mole/L}$		
1			1		
2			2		
3	1.32	1.31	3		
4	1.46	1.45	4	2.62	2.63
5			5		
6			6		
7	3.26	3.26	7	3.86	3.86
8	2.2	2.4	8	3.7	3.5
9	1.91	1.91	9	3.17	3.18
10	1.87	1.88	10	2.97	3.05
11	1.71	1.78	11	3.07	3.14
12	1.87	2.06	12	2.88	2.94
13	2.07	2.07	13	3.36	3.36
14	1.93	1.90	14	3.08	3.05
15	1.96	1.96	15	3.08	3.12
16	1.90	1.90	16	3.18	3.19
17			17		
18			18		
19	1.851	1.846	19	3.002	3.004
20			20		
21	1.79	1.79	21	3.20	3.22
22	2.03	2.02	22	3.02	3
23	1.99	1.99	23	2.95	2.95
24			24		
25	1.78	1.82	25	3.05	3.03

## Laboratory Results for Nitrite + Nitrate

Nutrient 2002			MOOS-1		
	$\mu\text{mole/L}$			$\mu\text{mole/L}$	
1			1		
2	16.1	16.2	2	25.1	25.2
3	15.67	15.73	3	23.94	23.81
4	14.5	15.1	4	24.5	23.8
5	17.0	17.1	5	26.1	26.2
6	15	15.1	6	23.6	23.8
7	16.7	16.9	7	25.7	26.2
8	17.0	17.0	8	28.3	28.3
9	13.7	13.8	9	21.2	21.4
10	15.60	15.51	10	23.65	24.26
11	13.42	13.5	11	22.28	22.07
12	11.6	11.69	12	18.1	20.1
13	15.86	15.86	13	24.64	24.64
14	17.2	17.0	14	25.4	25.5
15	14.6	14.5	15	21.4	21.9
16	14.32	14.47	16	23.80	23.83
17	14.99	14.18	17	23.25	23.10
18	14.85	14.99	18	23.35	23.42
19	10.16	10.12	19	22.96	23.12
20	17.5	13.785	20	25.14	25.28
21	24.86	24.70	21	35.74	35.70
22	14.95	15	22	23.62	23.3
23	16.26	16.29	23	25.26	25.25
24	13.6	15.1	24	23.8	23.4
25	15	15.2	25	23.9	24.31

Lab	Methods for Orthophosphate Determination
1	Samples were analyzed in a 10 cm cell on an LKB Ultraspec 4050 at 885 nm. Reagents- Ammonium molybdate, sulfuric acid, ascorbic acid, potassium antimony-tartrate (Strickland and Parsons, 1972)
2	Flow Injection Analysis, Lachat Method # 31-115-01-3-C
3	Nutrient analyses are performed on a Skalar SanPlus continuous-flow AutoAnalyzer. Phosphate is analyzed using a modification of the Bernhardt and Wilhelms technique. An acidic solution of ammonium molybdate is added to the sample to produce phosphomolybdic acid, then reduced to phosphomolybdous acid (a blue compound) following the addition of dihydrazine sulfate. The reaction product is heated to ~55C to enhance color development, then passed through a 50mm flowcell and the absorbance measured at 820m.
4	Orthophosphate reacts with molybdenum VI and antimony III in an acidic medium to form an antimonyphosphomolybdate complex. This complex is subsequently reduced with ascorbic acid to form a blue color. The reaction is accelerated by heating to 37°C. The absorbance is read at 880 nm or 660 nm using a low refractive index 5 mm flow cell.
5	Flow Injection Analysis; orthophosphorus by ascorbic acid reduction. Lachat method 10-115-01-1-A.
6	Soluble orthophosphate is determined by Technicon Industrial Methods No. 155-71. A single reagent stream combining an acidified solution of ammonium molybdate, antimony potassium tartrate and ascorbic acid forms a phosphomolybdenum blue complex.
7	All analyses are conducted using standard seawater methods as described in Strickland and Parsons. All samples are corrected for refractive index and background color effects by measuring the absorbance of a sample blank with no added reagents
8	Samples were analyzed using a LACHAT Quik Chem 8000 FIA Ion Analyzer. SM 4500 P-F (Molybdate/Tartrate/Ascorbic Acid Method)
9	Analyses were performed with a Lachat Instruments Quik-Chem Automated Ion Analyzer using the manufacturer's brackish water methods.

Lab	Methods for Orthophosphate Determination
10	Industrial Method 155-71W modified from Murphy, J., and Riley, J.P., A Modified Single Solution Method for the Determination of Phosphate in Natural Waters, Anal. Chim. Acta, 27, p.30, 1962. The automated procedure for the determination of orthophosphate in seawater from the formation of a phosphomolybdenum blue complex which is read colorimetrically at 880nm. An acidified solution of ammonium molybdate and antimony potassium tartate is used along with a solution of ascorbic acid.
11	Analyses were performed using the Bran-Lubbe TRAACS 800 instrument. Automated ascorbic acid reduction - EPA 365.1: Ammonium Molybdate and antimony potassium tartrate, in an acid medium, reacts with ortho-P to form antimony-phosphomolybdate complex. This complex is reduced by ascorbic acid to form a blue colored complex which is read colorimetrically at 660 nm.
12	Lachat QC8000 Autoanalyzer made by Zellweger Analytics using methods written by the Applications Group at Lachat Instruments. The phosphate method is based on reactions specific for the orthophosphate ion ( $\text{PO}_4^{3-}$ ) and covers a range from 0.03 to 2.00 $\mu\text{M}$ . The $\text{PO}_4^{3-}$ reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The ascorbic acid and molybdate reagents are merged on the chemistry manifold and the reagent stream is then merged with the carrier stream. The sample zone appears at the detector less than 10 sec after injection. The absorbance is proportional to the concentration of $\text{PO}_4^{3-}$ in the sample.
13	Automated EPA 365.1, Colorimetric SM 4500-P E
14	EPA Method 365.1 using a Bran Luebbe AutoAnalyzer 3.
15	Ascorbic acid and molybdate method. USEPA. Method No.365.1
16	ammonium molybdate and hydrazine method
17	Nutrients (nitrate, phosphate, silicate) will be analyzed using established colorimetric techniques using a segmented-flow autoanalyzer (e.g. Technicon AutoAnalyzer II).



Lab	Methods for Orthophosphate Determination
18	Ortho-phosphate reacts with ammonium molybdate and antimony potassium tartrate in acid medium to form an antimony-phospho-molybdate complex. This complex is subsequently reduced with ascorbic acid to form a blue color. The color is proportional to the phosphorus concentration. The developed color is measured at 880nm. EPA method 365.1
19	Phosphate in the samples was determined by reacting with molybdenum (VI) and antimony (III) in an acidic medium to form an antimonyphosphomolybdate complex. This complex was subsequently reduced with ascorbic acid to form a heteropoly blue and the absorbance was measured at 710 nm (Zhang et al., 1999). Concentrations were determined using an Alpkem Flow Solution Auto-Analyzer. The water used for the preparation of standards and wash solution was filtered seawater obtained from the surface of the Gulf Stream.
20	The parameters are ran on a Quik Chem 8000 automated ion analyzer.
21	A flow-injection based automatic analyzer
22	A Flow Solution IV from Alpkem/O.I. Analytical using modified methodology from Perstorp Analytical
23	AutoAnalyzer II from Technicon using methods provided by Technicon with a few minor changes. 155-71W Orthophosphates in water and seawater.
24	Samples were analysed using a Segmented Flow Analyser (Skalar San Plus) with spectrophotometric detection .
25	Technicon AutoAnalyzer II. Reagents - ascorbic acid, antimony potassium tartrate, ammonium molybdate, sulphuric acid Filter - 880 nm, Standards were made up with 3.7% NaCl; Sample wash was 3.7% NaCl.

Lab	Methods for Silicate Determination
1	Samples were analyzed in a 1 cm cell on an LKB Ultraspec 4050 at 810 nm Reagents-ammonium molybdate, sulfuric acid, oxalic acid, ascorbic acid (Strickland and Parsons, 1972)
2	Flow Injection Analysis, Lachat Method # 31-114-27-1-A
3	Nutrient analyses are performed on a Skalar SanPlus continuous-flow AutoAnalyzer. Silicate is analyzed using the technique of Armstrong, 1967. An acidic solution of ammonium molybdate is added to a seawater sample to produce silicomolybdic acid which is then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. Tartaric acid is also added to impede PO <sub>4</sub> color interference. The sample is passed through a 50mm flowcell and the absorbance measured at 820nm.
4	Silica in solution as silicic acid or silicate reacts with molybdate reagent in aqueous acid media to form silicomolybdic acid. The complex is reduced by stannous chloride to form heteropoly acid. The reaction is intensified by heating to 65°C. The absorbance is measured at 820 nm using a low refractive index 5 mm flow cell.
5	not determined
6	Dissolved silica is determined by Technicon Industrial Methods No. 186-72W, essentially that of Armstrong et al. (1967). The procedure is based on the reduction of silicomolybdate in acidic solution to molybdenum blue by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to eliminate the interference from phosphate.
7	not determined
8	Samples were analyzed using a LACHAT Quik Chem 8000 FIA Ion Analyzer. SM 4500 SI-F (Molybdate/Oxalic Acid/Ascorbic Acid Method)
9	Analyses were performed with a Lachat Instruments Quik-Chem Automated Ion Analyzer using the manufacturer's brackish water methods.

Lab	Methods for Silicate Determination
10	Industrial Method 186-72W modified from Strickland and Parsons, A Handbook of Seawater Analysis Determination is based on the reduction of silicomolybdate in acidic solution to 'molybdenum blue' by ascorbic acid. Oxalic acid is introduced into the sample stream before the introduction of ascorbic acid to eliminate interference from phosphates. Absorbance read 660nm.
11	Analyses were performed using the Bran-Lubbe TRAACS 800 instrument. Automated Molybdate reactive silica - Bran-Lubbe method # 785-86T; this method is referenced to USGSI-2700-85. In this method Oxalic acid is first introduced into the sample stream to remove phosphorous interference. Ammonium Molybdate in an acidic medium then reacts with silicate to form silicomolybdate. This complex is reduced by Ascorbic acid to a blue colored complex which is read colorimetrically at 660 nm.
12	not determined
13	Automated EPA AERP 22, Colorimetric SM 4500-Si F
14	EPA Method 370.1 using a Lachat QuikChem Autoanalyzer.
15	Bran & Luebbe autoanalyzer with Nap (Labtronics, Inc.) software. Ascorbic acid, molybdate, and oxalic acid method. Technicon method.
16	Armstrong et al (1967) method: ammonium molybdate, tartaric acid, and stannous chloride
17	Nutrients (nitrate, phosphate, silicate) will be analyzed using established colorimetric techniques using a segmented-flow autoanalyzer (e.g. Technicon AutoAnalyzer II).
18	Soluble silica species react with molybdate in acidic solution to form B-molybdosilicic acid. The B-molybdosilicic acid is then reduced by ascorbic acid to form molybdenum blue. The absorbance of the molybdenum blue, measured at 660 nm is linearly proportional to the concentration of silicate in the sample. Modified EPA Method 366.0

Lab	Methods for Silicate Determination
19	Silicic acid in the sample was reacted with molybdate in a acidic solution to form B-molybdosilicic acid, which was then reduced by ascorbic acid to form the molybdenum blue. Absorbances were measured at 660 nm (Zhang et al., 1997b) Concentrations were determined using an AlpKem Flow Solution Auto-Analyzer. The water used for the preparation of standards and wash solution was filtered seawater obtained from the surface of the Gulf Stream.
20	The parameters were run on a Quik Chem 8000 automated ion analyzer.
21	A flow-injection based automatic analyzer
22	A Flow Solution IV from Alpkem/O.I. Analytical using modified methodology from Perstorp Analytical
23	AutoAnalyzer II from Technicon using methods provided by Technicon with a few minor changes. 186-72W/B Silicates in water and seawater
24	not determined
25	Technicon AutoAnalyzer II system. Reagents - ammonium molybdate, oxalic acid, ascorbic acid Filter - 660 nm, Standards were made up by standard additions; Sample wash was 3.7% NaCl.

Lab	Methods for Nitrite Determination
1	not determined
2	not determined
3	Nutrient analyses are performed on a Skalar SanPlus continuous-flow AutoAnalyzer. A modification of the Armstrong (1967) procedure is used for the analysis of nitrite. Sulfanilamide is introduced to the sample stream followed by N-(1-naphthyl)ethylenediamine dihydrochloride which couples to form a red azo dye. The stream is then passed through a 50mm flowcell and the absorbance measured at 540nm.
4	Nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, which is proportional to the nitrite concentration. This reaction occurs at room temperature. The absorbance is measured at 540 nm using a standard 5 mm flow cell.
5	not determined
6	Determined by Technicon Industrial Methods No. 158-71W (revised August 1979) and is a modification of the Armstrong et al. (1967) procedure. Nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye.
7	All analyses are conducted using standard seawater methods as described in Strickland and Parsons. All samples are corrected for refractive index and background color effects by measuring the absorbance of a sample blank with no added reagents
8	Samples were analyzed using a LACHAT Quik Chem 8000 FIA Ion Analyzer. SM 4500 NO <sub>3</sub> -F (Buffer/Sulfanilamide/NED)
9	Analyses were performed with a Lachat Instruments Quik-Chem Automated Ion Analyzer using the manufacturer's brackish water methods.
10	Method is identical to Nitrate/Nitrite without the use of a copper-cadmium reduction column.

Lab	Methods for Nitrite Determination
11	Analyses were performed using the Bran-Lubbe TRAACS 800 instrument. Automated Diazotization - Bran-Lubbe method # 784-86T; this method is referenced to USGS I-4540-85. Same as Nitrate- Nitrite method except no cadmium reduction.
12	Lachat QC8000 Autoanalyzer made by Zellweger Analytics using methods written by the Applications Group at Lachat Instruments. The nitrite method is based on reactions specific for the nitrite ion ( $\text{NO}_2^-$ ) and covers a range from 0.02 to 5.0 $\mu\text{M}$ . Nitrite is determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The diazonium ion is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 520 nm.
13	Colorimetric EPA 353.2, Cadmium SM 4500-NO2 B
14	EPA Method 353.2 using a Lachat QuikChem Autoanalyzer.
15	USEPA Method 350.1
16	1-naphthylethylenedihydrochloride and sulfanilamide
17	not determined
18	not determined
19	Nitrite was determined by diazotizing with sulfanilamide and coupling with N-1 naphthyl ethylenediamine dihydrochloride to form an azo dye. The color produced is measured at 540 nm (Zhang et al., 1997a). Concentrations were determined using an AlpKem Flow Solution Auto-Analyzer. The water used for the preparation of standards and wash solution was filtered seawater obtained from the surface of the Gulf Stream.
20	not determined

Lab	Methods for Nitrite Determination
21	A flow-injection based automatic analyzer
22	A Flow Solution IV from Alpkem/O.I. Analytical using modified methodology from Perstorp Analytical
23	AutoAnalyzer II from Technicon using methods provided by Technicon with a few minor changes. 161-71W/B Nitrite in water and seawater
24	not determined
25	Nitrite and TOxN - were analyzed using the Technicon AutoAnalyzer II system. Reagents - ammonium chloride, sulfanilamide, N-1-naphthylethylenediamine dihydrochloride, phosphoric acid. Filter - 550 nm, Standards were made up with 3.7% NaCl; Sample wash was 3.7% NaCl.

Lab	Methods for Nitrite + Nitrate Determination
1	not determined
2	Lachat Method #31-107-04-1-C
3	Nutrient analyses are performed on a Skalar SanPlus continuous-flow AutoAnalyzer. A modification of the Armstrong (1967) procedure is used for the analysis of nitrate plus nitrite. For this analysis, the seawater sample is passed through a cadmium reduction column where nitrate is quantitatively reduced to nitrite. Sulfanilamide is introduced to the sample stream followed by N-(1-naphthyl)ethylenediamine dihydrochloride which couples to form a red azo dye. The stream is then passed through a 50mm flowcell and the absorbance measured at 540nm. The same technique is employed for nitrite analysis, except the cadmium column is not present, and a 50mm flowcell is used for measurement. Nitrate concentration is calculated by subtracting the nitrite value from the combined Nitrate + Nitrite (N+N) value.

Lab	Methods for Nitrite + Nitrate Determination
4	Nitrate is converted to nitrite by cadmium reduction. Nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, which is proportional to the nitrite concentration. This reaction occurs at room temperature. Nitrate is calculated by subtracting nitrite from nitrite+nitrate. The absorbance is measured at 540 nm using a standard 5 mm flow cell.
5	Flow Injection Analysis; cadmium reduction
6	Determined by Technicon Industrial Methods No. 158-71W (revised August 1979) and is a modification of the Armstrong et al. (1967) procedure. Nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye.
7	All analyses are conducted using standard seawater methods as described in Strickland and Parsons. All samples are corrected for refractive index and background color effects by measuring the absorbance of a sample blank with no added reagents
8	Samples were analyzed using a LACHAT Quik Chem 8000 FIA Ion Analyzer. Analyte methodology references used are as follows: SM 4500 NO3-F (Buffer/Sulfanilamide/NED/Cadmium reduction Method)
9	Analyses were performed with a Lachat Instruments Quik-Chem Automated Ion Analyzer using the manufacturer's brackish water methods.
10	Industrial Method 158-71W modified from (1) Armstrong, F.A.J., Sterns, C.R. and Strickland, J.D.H., 1967, Deep-Sea Res., 14, pp. 381-389, 'The Measurement of Upwelling and Subsequent Biological Processes by Means of the Technicon AutoAnalyzer and Associated Equipment'. (2) Grasshoff, K., Technicon International Congress, June, 1969. (3) Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, Nov. 1969. Nitrate is reduced to nitrite by a copper-cadmium reduction column. The Nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound couples with N-1-Naphthylenediamine dihydrochloride to produce a reddish purple azo dye. Absorbance band 550 nm.



Lab	Methods for Nitrite + Nitrate Determination
11	Analyses were performed using the Bran-Lubbe TRAACS 800 instrument. Automated cadmium reduction-EPA 353.2: sample in imidazole buffer pass through Cd column to reduce nitrate to nitrite. The total nitrite is now determined by diazotizing with sulfanilamide and coupling with N(1-naphthyl)-ethylenediamine dihydrochloride to form a colored azo dye complex, which is measured colorimetrically at 520 nm.
12	The nitrite + nitrate method is based on reactions specific for the nitrate ion (NO <sub>3</sub> <sup>-</sup> ) and Lachat QC8000 Autoanalyzer made by Zellweger Analytics using methods written by the Applications Group at Lachat Instruments. covers a range from 0.03 to 5.0 µM. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus the original nitrite) is then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 520 nm. Nitrate concentrations are obtained by subtracting nitrite values from the nitrite + nitrate values. All out of range samples are autodiluted by the autosampler and run again. Autodiluted values are reported for total oxidisable nitrogen; other values were within range for our manifolds.
13	Colorimetric EPA 353.2, Cadmium SM 4500-NO <sub>3</sub> B
14	EPA Method 353.2 using a Lachat QuikChem Autoanalyzer.
15	USEPA Method 350.1 Cadmium reduction
16	Cd column, 1-naphthylethylenedihydrochloride and sulfanilamide
17	Nutrients (nitrate, phosphate, silicate) will be analyzed using established colorimetric techniques using a segmented-flow autoanalyzer (e.g. Technicon AutoAnalyzer II).
18	Nitrate is quantitatively reduced to nitrite by metal cadmium in the column. Then the nitrite formed by reduction of nitrate plus nitrite originally present is determined as an azo dye at 540 nm following its diazotization by sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride. EPA Method 353.2

Lab	Methods for Nitrite + Nitrate Determination
19	Samples for nitrate analysis were passed through a on-line copperized cadmium column to reduce nitrate to nitrite for colorimetric determination (Zhang et al., 2000). Concentrations were determined using an AlpKem Flow Solution Auto-Analyzer. The water used for the preparation of standards and wash solution was filtered
20	The parameters are ran on a Quik Chem 8000 automated ion analyzer.
21	A flow-injection based automatic analyzer
22	A Flow Solution IV from AlpKem/O.I. Analytical using modified methodology from Perstorp Analytical
23	AutoAnalyzer II from Technicon using methods provided by Technicon with a few minor changes. 158-71W/B Nitrate & Nitrite in water and seawater
24	Samples were analysed using a Segmented Flow Analyser (Skalar San Plus) with Spectrophotometric detection.
25	Nitrite and TOxN - were analyzed using the Technicon AutoAnalyzer II system. Reagents - ammonium chloride, sulfanilamide, N-1-naphthylethylenediamine dihydrochloride, phosphoric acid. Filter - 550 nm, Standards were made up with 3.7% NaCl; Sample wash was 3.7% NaCl.